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Hematotoxicity, hepatotoxicity and nephrotoxicity in rabbits (Oryctolagus Cuniculus) after short term exposure to solvent (EGME)

Solvent toxicity

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Abstract
Aim: Exposure to chemicals spilled into different ecosystems represents a danger that threatens the survival of animal and plant populations. Currently, people need to use these products for industrial and / or domestic reasons without taking into account their effects on health and the environment.
This work aims to study the effect of a solvent: Ethylene glycol monomethyl ether (EGME) which belongs to the family of ethylene glycol ethers, on the proper functioning of the liver, blood and kidney.
Material and Methods: For this, two groups of animals were treated with EGME by gavage. The solvent EGME was applied in two doses: 50 ppm and 150 ppm for four successive weeks. Biochemical and Hematological parameters were studied.
Results: The results showed significant changes in biochemical parameters, characterized by a disruption of metabolism product (glucose, lipid, protein) associated with an increase in lipids (triglycerides and cholesterol), as well as disruption of alkaline phosphate levels in groups treated with EGME, increased levels of transaminases (GOT and TGP) and gamma GT in Group 2 (150 ppm) with a highly significant decrease in bilirubin in both groups. Markers of renal function (urea and creatine) were altered during the administration of EGME. Also, we found a significant increase in uric acid levels in both treated groups. Hematologically, there was a significant decrease in hemoglobin, red blood cell, white blood cell, and lymphocyte number, accompanied by an increase in platelet number in the EGME-treated groups. In the treated groups, there were decreased liver and testicular oxidative stress parameters (reduced glutathione) with lipid peroxidation MDA.
Discussion: According to the results, the administration of ethylene glycol monomethyl ether (EGME) to male rabbits Oryctolagus Cuniculus digestive at a concentration of 50 and 150 ppm causes functional disorders of many organs (liver, testis, kidney).

Keywords
Rabbit; Toxicity; Solvent; Ethylene glycol; Risk

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Introduction

Organic solvents present a double risk, on the one hand, due to their flammability and explosiveness, and on the other hand, obvious or insidious health risks, during accidental or prolonged exposure, whether deliberate or accidental. There is hardly any human activity that escapes them. They have become more and more necessary in several industrial processes, for example we quote: the manufacture of glues, paints, varnishes, inks, plastic [1].

Solvent toxicity has become a public health problem. Indeed, the Institute of Health and Safety at Work (ISST) has just launched a study on the impact of the use of solvents in the workplace. The toxicity of solvents is very divers and affects different organs and systems (ISST. Institute for Occupational Health and Safety. Occupational risk assessment, 2016).

- The central nervous system (toluene, xylene, trichlorethylene, tetrachlorethylene ...). Action on the CNS can cause addiction.
- The peripheral nervous system (hexane, petroleum ether, ...).
- The liver (Trichlorethylene, Tetrachlorethylene, ...)
- The kidneys (Styrene ...).
- The hematopoietic system (benzene, ethylene glycol, ...)

The toxicity of solvents may also interest other organs such as the respiratory tract, cardiovascular system, skin and the reproductive function, not to mention the carcinogenic action of some of them (benzene). For lighting and other measurable physical nuisances, solvents are the most commonly encountered, unknown, and underhanded abusers (Khayati N. Risk assessment of exposure to solvents in the leather processing sector (about 84 cases). University of Tunis El Manar, 2004).

Known since the 1930s, glycol ethers are a family of more than eighty representatives. Following early toxicological studies conducted in the 1970s, the safety of glycol ethers has been questioned. Since then, their toxicological profile has been studied in more detail. Work on animals has certainly identified target organs and potentially disturbed functions. However, transposition in humans remains difficult.

However, understanding the mechanisms of cellular and molecular actions can facilitate the assessment of the impact of long-term exposure to these products on human health. Nevertheless, in the current state of knowledge, the real risk of chronic toxicity in humans is limited to a set of presumptions derived from epidemiological studies in the workplace [2].

Glycol ethers are oxygenated solvents that have been widely used over the last thirty years. They constitute a diverse family of more than 30 different substances divided between those derived from ethylene glycol and those derived from propylene glycol [3].

Ethylene glycol is a colorless, odorless and relatively non-volatile liquid. It has a low vapor pressure and is completely miscible with water. According to short- and long- term oral studies on laboratory animals, the kidneys are the main target of ethylene glycol exposure [4].

Non-neoplastic degenerative changes have been consistently observed in the kidneys of several species at the lowest doses. According to a very large database, ethylene glycol has slight reproductive and developmental effects, including teratogenicity, in orally exposed rodents, although at doses greater than those associated with effects on the kidneys [5].

Since the first publication of Nagano in 1979, numerous studies on laboratory animals have shown that glycol ethylene glycol ethers (mainly methyl ether or EGME) have adverse effects on reproduction and reproduction development [6].

The objective of this work is to evaluate the deleterious effects of a solvent: ethylene glycol monomethyl ether (EGME) on some indicators of good health in the male rabbit Oryctolagus Cuniculus.

Material and Methods

The experimental work was approved by the Ethics Committee of the Health Department of the Wilaya El-Tarf, and validated by the Scientific Committee of the Biology Department at the El-Tarf University.

This study aims to illustrate the effects of a solvent that belongs to the family of glycol ethers on hematologic, hepatic and nephrotic profiles.

1. Biological material

The animals used in this study were male rabbits Oryctolagus cuniculus, widely used in various fields of research.

Classification of the animal:

- Animal Kingdom
- Branching of vertebrates
- Class of mammals
- Order of the Lagomorphs
- Leporidae family
- Genus Oryctolagus
- Species Oryctolaguscuniculus

Breeding conditions

Our study was conducted on 12 mature male rabbits of a local breed, these rabbits were aged between 5 and 9 months and weighed between 1400 and 1900g at the beginning of the experiment.

These animals have been subjected to a period of adaptation to the conditions of the pet shop, 10 days approximately at room temperature (25 °), and a photoperiod of 12 h/12h.

The rabbits were grouped into three metal cages (50x60x53 cm), screened, provided with water troughs, each containing four rabbits and lined with a thick layer of wood chips renewed three times per week. The cages were cleaned daily until the end of the experiment.

Rabbits were fed 3 times a day, the diet consisted of vegetables (carrot, salad, cucumber) and dry food consisting of maize, barley and vitamin supplement. They had free access to water and food.

2. Chemical equipment

The solvent used in this experiment is ethylene glycol monomethyl ether (EGME) which belongs to the category "E" of glycol ethers or the category of derivatives of ethylene glycol.

EGME (ethylene glycol monomethyl ether) is mainly used as a solvent for the manufacture of paints, varnishes, inks, dyes. It is also used as antifreeze for aviation fuels and as a cleaning and degreasing agent.

The substance is classified according to the data of the National Institute for Research and Security, Database (Ethylene glycol. Toxicological sheet. 2016; 25:3) as follows:

T: Toxic

R60: May impair fertility.
R61: Risks of adverse effects in children during pregnancy.
R20 / 21/22: Harmful by inhalation, in contact with skin and if swallowed.

3. Experimental protocol

After the adaptation period, the 12 rabbits were divided into three batches, each batch comprising four rabbits (n = 4) kept under the same conditions.

The experiment consisted of administering 2 increasing doses of ethylene glycol monomethyl ether (EGME) to rabbits: 50 ppm and 150 ppm as follows:

Control group: control rabbits (T) which receive only water.
Lot 1 (D1): Rabbits treated with EGME at 50 ppm.
Lot 2 (D2): Rabbits treated with EGME at 150 ppm.

The product was administered by gavage once a day (1ml), 5 days a week, for 4 successive weeks.

Sacrifice and blood collection and organs

After 30 days of treatment rabbits from 3 lots were fasted for 15 hours and then sacrificed (by decapitation), the blood was immediately collected in two tubes. The first contained EDTA anticoagulant and the second contained heparin, the latter was centrifuged at 3000 revolutions for 15 minutes for the determination of biochemical parameters (glycemia, urea, creatinine, Transaminases (TGO: transaminase glutamooxaloacetic, TGP: pyruvic glutamic transaminase), uric acid, albumin, BT: total bilirubin, GGT: gamma-glutamyl transferase, alkaline phosphatase, cholesterol, triglycerides). The plasma obtained was stored at a temperature of -20 ° C.

The blood was put in the other tube containing EDTA for the determination of hematological parameters.

After dissection, the liver and testes were carefully removed, stripped of adipose tissue, rinsed in 0.9% NaCl sodium chloride solution. These organs were stored in the freezer for the determination of oxidative stress parameters: tissue protein, reduced glutathione (GSH), malondialdehyde (MDA).

Results

1. Variation in renal, hepatic and metabolic parameters

The urea level increased in the 50 ppm EGME group and decreased in the 150 ppm group compared to the control group. These variations were statistically significant ($p<0.05$). Our results also showed that there was a non-significant decrease ($p>0.05$) in creatinine levels in both treatment groups compared to controls. Thus, there was a decrease in the albumin level in the batch treated at a dose of 50 ppm and an increase in the batch treated at the dose of 150 ppm compared to controls. These variations were also non-significant ($p>0.05$). We recorded a non-significant increase ($p>0.05$) in uric acid level in the EGME-treated groups compared to controls.

There was a significant increase ($p<0.05$) in the activity of transaminases TGO, TGP and alkaline phosphatase in the groups receiving a dose of 150 ppm compared with the batch treated with dose 1 and the control, a significant decrease ($p<0.05$) in the total bilirubin level in 2 groups treated with EGME compared to controls. GGT levels were higher in the 2 treated groups compared to controls.

We found a non-significant ($p>0.05$) increase in glucose, cholesterol and triglyceride levels in two EGME-treated groups compared to controls (Table 1).

2. Variation in hematological parameters

The results show a non-significant decrease ($p>0.05$) in the number of white blood cells and lymphocytes with a non-significant increase ($p>0.05$) in platelets number in the two groups treated with EGME. The number of red blood cells showed a non-significant decrease ($p>0.05$) in the treated groups and a significant decrease ($p<0.05$) in hemoglobin levels in EGME-treated groups compared to controls (Table 2).

3. Effect of EGME on oxidative stress biomarkers (glutathione GSH level) and lipid peroxidation (Variation in malonaldehyde MDA) in the liver and testis

We noted a significant decrease ($p<0.05$) in glutathione levels, and a highly significant increase ($p<0.01$) in malonaldehyde MDA (the potential of lipid peroxidation) in the liver and testis in EGME-treated groups compared to the control (Table 3).

Table 1. Variation in renal, hepatic and metabolism parameters

	Controls	Dose 1 (50 ppm)	Dose 2 (150 ppm)
Urea (g/l)	0.41	0.61	0.37
Creatinine (g/l)	12.33	11.60	12.08
Albumin (g/l)	31.06	28.87	31.20
Uric acid (mg/l)	1	2.75	2.25
TGP (µl/l)	38.35	35.60	48.42
TGO (µl/l)	32.65	19.82	38
alkaline phosphatase (µl/l)	135.30	116	138.2
Total bilirubin (µ/l)	15.42	4.03	4.34
GGT (µ/l)	10	5.5	12.5
Glucose (g/l)	0.85	0.98	1.04
Cholesterol (g/l)	0.50	0.58	0.85
Triglycerides (g/l)	0.51	0.53	0.75

Table 2. Variation in hematological parameters

	Controls	Dose 1 (50 ppm)	Dose 2 (150 ppm)
White blood cells ($x10^3$/ml)	12.57	11.87	7.20
Lymphocytes ($x10^3$/ml)	5.22	2.82	3.17
Platelets ($x10^3$/ml)	133.75	170.5	214.5
Red blood cells ($x10^6$ /ml)	5.67	3.91	4.05
Hemoglobin level (g/dl)	12.52	6.65	7.35

Table 3. Variation in glutathione GSH and malonaldehyde MDA levels

	Controls	Dose 1 (50 ppm)	Dose 2 (150 ppm)
Glutathione GSH level in liver (nM/mg protein)	4.32	2.62	2.02
Glutathione GSH level in testis (nM/mg protein)	3.12	1.12	0.03
MDA level in liver(µmol/mg protein)	0.10	0.54	1.22
MDA level in testis (µmol/ mg protein)	0.60	3.02	7.34

Discussion

Most chemicals for industrial use have toxic effects on the indicators of good health in humans and animals. Ethylene glycol monomethyl ether, as an example of a study, exerts its toxicity via acid metabolites and even more aldehydes. These are able to penetrate the cell's nucleus and alter the structure and function of the genome governing growth and cell development.

Through our results, ethylene glycol monomethyl ether (EGME) affects certain biological functions in male rabbits, by ingestion, altering the liver, testes and kidneys following the action of oxidative stress.

The effect of this solvent on liver function manifests itself at two levels: metabolic and structural. All products of physiological metabolism (glucose, lipid, protein) are disrupted in the treated groups. This effect is well documented in several studies at the national level (Djabali N. Effects of a solvent: Ethylene Glycol Monomethyl Ether (EGME) on male fertility and some biochemical and cellular parameters of the blood in the rabbit Oryctolagus cuniculus, Badji Mokhtar Annaba University; 2011) and international [7].

We noticed a disruption of glucose and protein (albumin) associated with an increase in lipids (triglycerides and cholesterol). All these disturbances are due to alteration in liver functions by EGME metabolites (aldehyde function) that can inhibit oxidative phosphorylation, glucose metabolism (glycolysis and Krebs cycle, main producers of ATP), protein synthesis, DNA replication and ribosomal ARNa. In the same context, EGME mainly causes metabolic acidosis (accumulation of acidic products e.g glycolic acid, oxalic acid and lactic acid). In addition, there is an increase in the blood concentration of lactic acid contributing to lactic acidosis. The formation of acidic metabolites also causes inhibition of other metabolic pathways such as oxidative phosphorylation [8]. With respect to other biochemical metabolites, triglycerides and cholesterol appear to be affected by EGME. The administration of the solvent in both the 50 and 150 ppm groups induces an alteration of the lipid profile, which is manifested by an increase in the level of cholesterol and plasma triglycerides [9].

This result is consistent with Benjedou's study of rabbits treated with dermal EGME. All these disturbances are due to the alteration of liver functions by EGME metabolites [7].

A significant increase in cholesterol concentration in the treated groups compared to the control is associated with liver tissue damage, which may be due to an imbalance in the enzymes responsible for the conversion of cholesterol to male sex hormones, which causes a decrease in testosterone concentration [10,11].

The results obtained show that there is an increase in the alkaline phosphatase level in the treated groups (150 ppm), this increase is due to the excess of iron in the body, this effect is dose-dependent (significant increase in group II). Our results are consistent with Adams' (1991) investigations, which indicate that excess iron induces an increase in alkaline phosphatase activity. These enzymes are normally contained in the cells of the liver. If the liver is injured, liver cells reverse enzymes in the blood; the level is increased in cases of liver cell death [11-13]. As well as an increase in alkaline phosphatase activity results from liver and bone dysfunction (Ait Hamadouche. Effects of chronic lead exposure on the reproductive system and the hypothalamic-pituitary axis in male Wistar rats: Histological and biochemical study. University of Es-Senia Oran, 2009;171).

In our study, we found a significant increase in transaminase levels (TGO and TGP). This effect is dose-dependent. Transaminases are essential enzymes in cytolysis [13]. They pass into the serum in case of hepatic or muscular cytolysis, a significant increase is observed in the cytolysis of toxic hepatitis [14].

The results show that there is a significant decrease in bilirubin in both groups treated with EGME. This decrease may be due to liver and gall bladder dysfunction, probably a problem in the stage that allows the excretion of bilirubin in the bile [15].

Our results also revealed an increase in the gamma GT level in the 150 ppm-treated group. An increase in serum gamma GT concentration is a good indicator of the involvement of bile duct epithelial cells (Dridi N, Segueni N. Study of the antitoxic effect of the methanolic extract of the Cotulacinarea species with respect to the pesticide Chlorpyriphos in albino wistar rats. Echahid Hamma Lakhdar University of El-Oued) 2015)

Conclusion

According to the results, the administration of ethylene glycol monomethyl ether (EGME) to male rabbits Oryctolagus Cuniculus digestive at a concentration of 50 and 150 ppm causes functional disorders of many organs (liver, testis, kidney). Our results seem very useful to confirm the harmful effects of this solvent:

- Hematotoxicity is expressed by an effect on the bone marrow
- Hepatotoxicity is manifested by the effect on enzymes and metabolism products.
- Alteration of the detoxifying potential (glutathione reduced GSH).
- Altered markers of renal function.
- Hepatic and testicular oxidative stress; Lipid peroxidation in the liver and testis.

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Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

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